Table 1

Feeding cues for *Buccinium undatum.*

		# Attempts to feed	# No attempts to feed
Effect of hunger	2 weeks starvation	0	18
	6 weeks starvation	5	3
Feeding stimuli	MgC12 *Mytilus* treatment	0	5
	Healthy *Mytilus*	0	12
	Nicked tissue *Mytilus*	4	4
Effect of prey-specific olfactory stimuli		Arousal	No arousal
	Mytilus extract	4	0
	Seaweed extract	0	8

Whelks' responses were scored in four categories: no attempt to feed, unsuccessful attempt, successful attempt, and "arousal," which was indicated by a major postural change or locomotion in response to a stimulus.

Experiment A—Effect of Hunger

Whelks (n = 26) were each isolated into 9.45 L aquariums filled with filtered seawater. Each aquarium had an air stone. Whelks were fed once with a pre-opened, medium-sized (relative to the whelk) *M. edulis,* which they consumed. They were then kept without food for 2 or 6 weeks before the feeding trial. The effect of hunger was then examined by presenting individual whelks with a live, healthy *M. edulis,* and observing them for 2–3 hours for any attempts to feed.

Experiment B—Effect of Prey Condition

In this experiment, whelks that had not been fed for 3 weeks were either given a live, healthy, normal *Mytilus* or one that had been weakened using a 3.5% solution of $MgCl_2$ in seawater. The mussels were placed in the treatment solution for 15 minutes. This treatment affected the nervous system of the mussels, so that when touched or handled they remained in an open relaxed position and did not close their valves. This condition lasted 30 minutes, which was sufficient for the experiment. Outcome was scored in the same fashion as in experiment A.

I examined responses of whelks that had not been fed for 3 weeks that were given healthy mussels, and whelks given mussels with slight tissue damage. The mussels were nicked slightly on their mantle with a razor, but their adductor muscles were left intact. Outcome was again scored in the same fashion as in experiment A.

Experiment C—Effect of Chemical Cues

Chemoreception is the primary means by which whelks locate food (Brock, 1936). To identify the source of olfactory cues that induce feeding, I used mussel "scent." To produce mussel scent, a mussel was opened with a razor and placed in 25 mL of distilled water for approximately 20 minutes. Seaweed "scent" was used as a control and was created by crushing a few grams of *Laminaria* sp. in 25 mL of distilled water. Whelks were not fed for 3 or 5 weeks before testing. Whelks were placed in the center of their respective tanks. Next, 1 mL of either mussel "scent" or seaweed (*Laminaria* sp.) extract was added to the water in the vicinity of the whelk. Since no actual prey items were present during this experiment, whelk responses were scored as either "arousal" or no response. Aroused whelks showed an immediate postural change often followed by locomotion directed into the current and toward the scent plume. Unresponsive whelks remain stationary and relaxed with their shell resting upon the substrate.

RESULTS

Experiment A: Whelks (n = 18) deprived of food for only 2 weeks never attempted to feed, whereas those (n = 8) starved for 6 weeks were significantly more likely to attempt to feed on healthy mussels (Fisher's exact test $P = 0.001$, Table 1).

Experiment B: Whelks made no attempt to feed on mussels subjected to a $MgCl_2$ treatment (n = 5) or untreated mussels (n = 12). However, when offered mussels that had sustained mantle tissue damage (with the adductor muscles still intact), significantly more whelks attempted to feed (n = 8) (Chi-square goodness-of-fit test $P = 0.002$, Table 1).

Experiment C: Whelks showed a significant response to prey-specific odors. All showed a typical arousal response to mussel scent (n = 4), and none did to seaweed scent (n = 8) (Fisher's exact test $P = 0.002$, Table 1).

DISCUSSION

Behavior is contingent upon both the external conditions and the internal state of the animal. In foraging, the decisions made regarding when and how to feed often re-

flect the hunger of the animal—the internal variable, and the food availability—the external variable. In this case, whelks preferred damaged prey, but when faced with the risk of starvation as a result of restricted food availability, there was a shift in the foraging decision. That is, the hungry whelks were more likely to attempt to open a healthy mussel.

Nielsen (1975) showed that healthy mussels have an excellent defense against whelk predation in their ability to close their valves tightly for long periods of time. Opening an intact mussel is energetically expensive and presents a risk of injury. Thus feeding upon a healthy bivalve does not constitute as large a net energy gain as feeding upon a damaged bivalve. This suggests two things: first, injured prey would be preferred to intact prey; and second, the whelk would attempt to feed on a healthy bivalve only as the risk of starvation increases. Both of these predictions are supported by the data.

The inverse relationship between hunger and selectivity is widespread. Feeding preferences of another predatory gastropod, the dogwhelk *Nucella lapillus* (Linnaeus, 1758), have also been shown to be influenced by starvation. Dogwhelks restricted from feeding were more likely to feed upon a patch of barnacles (Vadas et al., 1994). Likewise, a study of the predatory snail *Acanthina spirata* (de Blaineville, 1832) showed the species to be less selective between two different barnacle species when starved (Perry, 1987).

The behavior of *Buccinum undatum* can be fitted to a risk-sensitive model of behavior. Here, the term risk refers to probabilistic variation in prey (Stephens & Krebs, 1986). Caraco et al. (1980) demonstrated that an animal's energy budget can predict whether it is risk-averse or risk-prone. That is, an animal on a negative energy budget (a hungry whelk, for example) will prefer variable food rewards. In this case, a variable food reward is a healthy mussel. An attempt to open it will result in either a large gain in energy or none at all. On the other hand, an animal on a positive energy budget will prefer less variable food rewards—in this case, a damaged mussel for which there is no chance of failure if the whelk attempts to feed. Stated simply, hungrier animals are more impulsive and thus less selective with regards to foraging opportunities (Synderman, 1983). It is possible that starvation, in addition to reducing the selectivity of whelks, has a negative effect on the successes of attacks upon bivalves. If this is the case, there may come a point at which prey selectivity increases again, as any further attempts to open healthy bivalves would prove unsuccessful. An investigation on the effects of starvation and diet of another species of whelk, *Bullia digitalis*, demonstrated that starvation had a detrimental effect upon the feeding process in some cases (Stenton-Dozey et al., 1995).

The cues a whelk uses to make a decision to feed are important. Odor cues from damaged mussel tissue are sufficient to trigger feeding behavior, whereas odor cues from undamaged tissue are not. Although the mussels treated with $MgCl_2$ were defenseless, no whelks attempted to feed, even after investigating the mussel and encountering no defensive response. However, damaged mussels that could still close tightly were attractive prey. Chemosensory cues from the damaged tissue are important to stimulate feeding behavior, whereas visual or tactile cues seem to be of little importance. When mussel scent alone was presented, all whelks showed the postural change associated with the stimulus of the mussel extract that suggested interest in feeding. None showed any interest or response to an equivalent addition of seaweed extract to the water, demonstrating that whelks distinguish prey odors from non-prey odors in the water, and react accordingly.

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Larval Development, Precompetent Period, and a Natural Spawning Event of the Pectinacean Bivalve *Spondylus tenebrosus* (Reeve, 1856)

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Abstract. The artificial induction of spawning, and the development of larval *Spondylus tenebrosus,* a spondylid pectinacean bivalve, is described. A combination of warming and injections of serotonin into the adductor muscle of these animals produced spawning within 1.5 hours. Larvae were cultured at temperatures ranging from 22–24°C. Pediveligers were first observed within 12 days of fertilization, and settlement was first observed 21 days after fertilization. Pediveligers that were not allowed to settle were healthy 60 days after fertilization, when culture ended, indicating that larval *S. tenebrosus* can delay settlement, and remain planktonic for at least 2 months. In addition, a spawning event of natural populations on the southern shore of Oahu, Hawaii was indirectly observed. This spawning event occurred during a period of maximum annual temperature, suggesting that warming may be a natural spawning cue for *Spondylus tenebrosus.*

INTRODUCTION

Spondylus tenebrosus (Reeve, 1856), a spondylid pectinacean bivalve, is a commonly recruiting bivalve in Hawaiian coastal waters (Bailey-Brock, 1989). *S. tenebrosus* occurs in Australia, and the Gilbert, Marshall, and Hawaiian Islands (Kay, 1979). In Hawaii, it mainly occupies vertical surfaces of natural and artificial reefs, and the upper surfaces of holes and small caves (Thorsson, 1987). It occurs from the shallow subtidal zone to at least a depth of 40 meters.

Pectinacean larval development is similar to that observed in many bivalves (Cragg & Crisp, 1991). Gametes are spawned into the water column, and fertilization is external. Cleavage stages develop into mobile ciliated trochophores. The first shelled stage is prodissoconch I, and it is during this stage that the larvae first feed on phytoplankton. Prodissoconch II (umbone) larvae are characterized by the initiation of umbone development and the appearance of commarginal growth lines. The pediveliger stage, identified by the presence of a foot, is competent to settle. Pediveligers may leave the water column and crawl on the bottom using this foot prior to settlement.

The larval developmental period of pectinaceans, as observed in previous lab studies, varies with temperature and salinity, and among species. Delayed growth and development of pectinacean larvae have been observed at decreased temperatures (Beaumont & Budd, 1982). Pectinacean precompetent periods range from 14 days, for the tropical pectinacean *Amusium pleuronectes* (Belda & Del Norte, 1988), to more than 42 days for the temperate pectinacean *Chlamys hastata* (Strathmann, 1987).

Although larvae of *Spondylus tenebrosus* are common in the Hawaiian coastal plankton throughout the year, the larval development of this species has not been previously described. The period between the initial release of gametes and the development of larvae that are capable of settlement is referred to here as the precompetent period. The goal of the present study was to determine the minimum precompetent period of *S. tenebrosus,* and thus determine the minimum period that larvae are planktonic and subject to dispersion by circulation. This information was needed to supplement a field study of benthic invertebrate recruitment pattern forced by the interaction of circulation, larval planktonic period, and adult distribution (work in progress).

MATERIALS AND METHODS

Broodstock

Adult *S. tenebrosus* were collected twice from an artificial reef off Waikiki Beach (southern shore of Oahu, Hawaii) at a depth of 35 meters using SCUBA. Six individuals were collected on 10 August 1996 and were dissected immediately to check for gonad condition. Five individuals had ripe gonads (four females and one male). This initial collection was conducted to determine if animals were available and, if so, to check their gonad condition. A second collection was conducted after preparations were made to culture adults and larvae. Sixteen adults were collected on 21 September 1996 for broodstock. These animals were transported to the lab in a 128 L cooler, half filled with surface water from the collection

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site (T = 26.7°C). The animals were cleaned of fouling algae and invertebrates using a plastic scrubbing pad and a putty knife. They were then placed in a 70 L aquarium filled with seawater at ~26°C. The aquarium temperature was kept constant until spawning induction was attempted 2 hours later.

Spawning

The broodstock was placed in a 3 L glass bowl filled with 0.2 μm filtered seawater (FSW) for spawning (salinity ≈ 34). Spawning induction was first attempted by injection of 0.5 mL of a 2 mM serotonin solution (Strathmann, 1987; Monsalvo-Spencer et al., 1997; Rhee & Davis, 1997). Serotonin was injected through ~2 mm holes that were drilled through the anterior valves so that adductor muscles could be injected; one hole was drilled per animal. Injections were not possible without drilling because the animals rapidly closed their valves upon sensing any motion. Several animals opened and closed their valves quickly within 2 minutes of injection, but no gametes were seen exiting the mantles. No spawning was observed for 2 hours. The animals were then warmed from 26.2°–35.0°C over a 1-hour period (salinity ≈ 34) as a second attempt to induce spawning; spawning did not occur. Five individuals were then dissected to obtain gametes, but all gonads were empty. It was therefore likely that the *S. tenebrosus* population on the artificial reef off Waikiki had spawned since the first collection of 10 August.

Two different populations of *S. tenebrosus* in Mamala Bay were then sampled on 23 September to check for gonad condition. Five animals were taken at a depth of 3 meters from a sunken barge off the west end of Waikiki Beach, and five animals were collected at a depth of 30 meters from a natural reef off Ewa Beach. These animals were dissected upon return to the lab, and none had ripe gonads.

The 11 individuals that remained from the 21 September collection were maintained in a 60 L aquarium filled with natural seawater at ~26°C. The animals were fed a mixed diet of phytoplankton in an attempt to return them to spawning condition. The algal diet was composed of dense suspensions (algal concentrations were not quantified) of *Isochrysis galbana* (Tahitian strain) and *Skeletonema costatum* (Greville) at a temperature of ~26°C. On 21 November (60 days later), the animals were reinjected with serotonin solution as before and were monitored for 2 hours. No spawning occurred. Warm shock was then attempted as before. After warming, water in the spawning pan was allowed to cool. Several individuals spawned within minutes of each other, beginning approximately 1.5 hours after warming began. It was not possible to determine how many animals had spawned due to the turbidity created by spawning. The temperature in the pan was 28.3°C when the animals spawned.

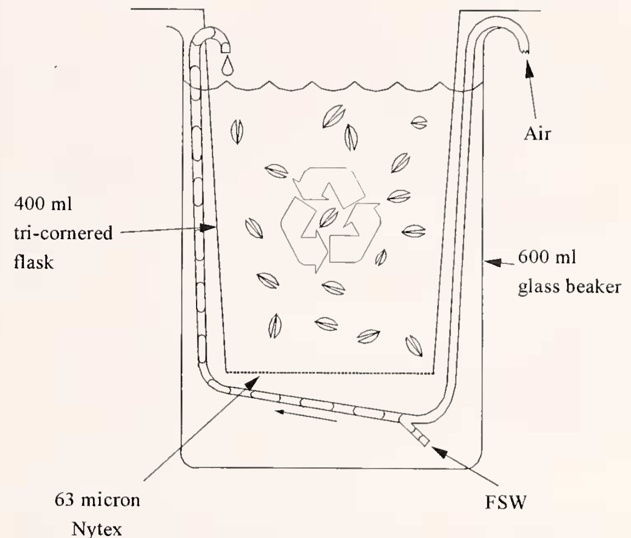

Figure 1. Diagram of Airlifted-Droplet Stirrer vessel. Droplets are lifted from bottom of vessel by air passing Y-tube at bottom. Larvae are kept well mixed within vessel because (1) surface tension is disturbed which prevents positively buoyant larvae from aggregating at the surface, and (2) turbulence is created by circulation within the vessel.

Larval Culture

Water was removed from the spawning pan, sieved through cheesecloth, and transferred into shallow glass bowls. A compound microscope was used to measure the largest dimension of 50 fertilized eggs removed from the spawning pan with a disposable pipette. The fertilized eggs that remained were then removed from the spawning pan using a pipette under a dissecting microscope and placed in 10 sterilized 500 mL glass beakers containing 0.2 μm FSW. Filtered seawater was changed three times to decrease the concentration of pathogens. Streptomycin sulfate and penicillin-G sodium were added to the larval culture media as antibiotics, initially at 50 $\mu g\ mL^{-1}$ for each, but concentrations were doubled 3 days after spawning due to high larval mortality (estimated at > 75%). Developmental stages of 20 haphazardly chosen live larvae from three randomly chosen beakers were noted at 1 to 3 hour intervals for the first 48 hours. After that, at least 200 larvae were placed in each of 12 600 mL culture vessels. The culture vessels were continually stirred using air to lift water from the bottom of the vessel and return it at the surface (Figure 1). These larval culture vessels were designed by Michael Hadfield, and are described by Strathmann (1987:16). This design reduces the number of larvae stuck at the water's surface. Twenty haphazardly chosen larvae were removed from three randomly chosen culture vessels every 2 to 3 days for measurement of the largest dimension and determination of developmental stage. This method of determining larval size and developmental stage was used to minimize in-

Table 1

Numerical codes of developmental stages for descriptive statistical analysis.

Larval stage	Numerical code
Fertilized egg	0
First Cleavage (includes all cleavage stages)	1
Blastula	2
Gastrula	3
Trochophore	4
Prodissoconch I (Straight-Hinged)	5
Prodissoconch II (Umbo Veliger)	6
Pediveliger	7

dividual larval handling. The number of culture vessels decreased as larvae died or settled and larvae from different culture vessels were combined. Developmental stages were numerically coded for descriptive statistical analysis (see Table 1). Salinity in the vessels was approximately 34, and the temperature varied between 22° and 24°C. Vessel seawater was changed every 1 to 2 days, and dead or abnormal larvae were removed.

Larval Feeding and Algal Culture

Larvae were fed dilute suspensions of *Isochrysis galbana* and *Skeletonema costatum* (log-growth stage) beginning when larvae reached the first feeding stage (straight-hinged stage) 24 hours after fertilization. The concentrations of algae in the feeding suspensions were not determined. However, it was apparent that the larvae were feeding because visual inspections showed their guts were full of algae. Enough algae were added to the vessels to keep larvae guts full throughout the culture period. *Isochrysis galbana* and *S. costatum* were cultured in autoclaved f/2 medium-enriched seawater (Bidwell & Spotte, 1985:305) under continuous illumination by a cool white fluorescent light in aerated culture flasks.

Settlement

Shell fragments of adult *S. tenebrosus* were placed into three randomly chosen larval culture vessels after the first pediveligers appeared in the samples in an attempt to induce settlement. The shell fragments were obtained from adults that were crushed minutes before the settlement experiments began. Larvae were not counted in settlement vessels; instead, the cumulative number of settlers was counted on the days that developmental stage and size data were recorded. Cumulative counts of settlement were approximate due to the complex surface topography of shell fragments. Shell fragments were not added to the remaining culture vessels in order to determine the ability of *S. tenebrosus* to remain competent for prolonged larval periods. Shell fragments were finally added to these vessels 2 days before the end of the culture experiment. Larvae were cultured for a total of 60 days after fertilization.

Field Temperature Record

Warming is a spawning cue for many species of bivalves (Strathmann, 1987) and pectinaceans specifically (Cragg & Crisp, 1991). Therefore, an oceanic temperature record was needed to determine if a warming event occurred when *S. tenebrosus* spawned in Mamala Bay between 10 August and 21 September. However, no temperature record was available for the Bay. Alternatively, a temperature record from a C-MAN (Coastal-Marine Automated Network) buoy 51026 (21°21′06″N, 156°55′54″W), located 17 km north of Molokai and ~ 90 km from Mamala Bay, was obtained from the National Oceanographic Data Center's online archive. The buoy temperature record provides a useful indicator of regional warming events lasting several days for the region that includes Molokai and Oahu. The NODC C-MAN buoy 51026 temperature record contains hourly data. These data were resampled at a daily frequency after lowpass filtering with an eighth order Chebyshev type I lowpass filter to remove short-term (e.g., tidal period) variations.

Data Analysis

Statistical testing of growth and developmental data was problematic since different larvae from different culture vessels were measured over the course of the experiment (this was done to minimize handling of individual larvae). Therefore, it was necessary to assume that larvae from all culture vessels formed one population. In order to lump data among culture vessels for time series of size and development, it was necessary to test for differences in these parameters among culture vessels. The null hypothesis was that the sizes and developmental stages of larvae were not different among culture vessels each day that measurements were conducted. This hypothesis was tested using the non-parametric Kruskal-Wallis test (Sokal & Rohlf, 1969). No significant differences among culture vessels for either size or developmental stage were observed ($\alpha = 0.05$). Therefore, larval size and developmental stage data were pooled among culture vessels within each day that measurements were conducted. The means and standard deviations for each parameter were then plotted as a function of time.

RESULTS

Natural Spawning in Mamala Bay

A warming event occurred in late August and early September (Figure 2), which may have triggered *S. tenebrosus* in Mamala Bay to spawn sometime between 10 August and 21 September 1996, when adults were collected. Water temperatures increased from 24.4° to 26.4°C

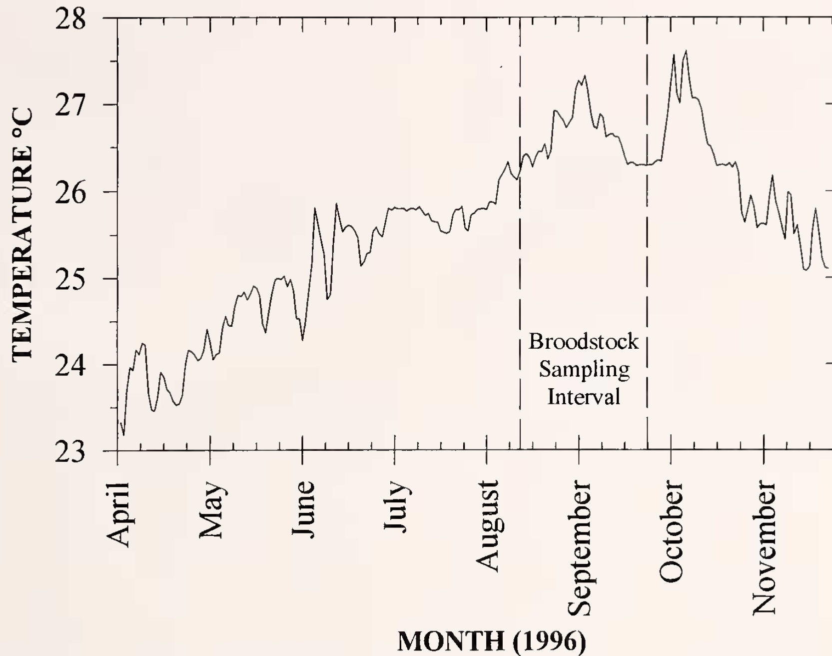

Figure 2. Temperature data from C-MAN buoy 51026 (17 km north of Molokai). Dotted lines indicate broodstock sampling dates.

from early June to 10 August. Temperatures then increased at a faster rate from 26.4°C, on 10 August, to a maximum of 28.5°C, on 31 August. Water temperatures decreased after the warming event at a rate similar to the rate of increase prior to the 31 August maximum; the temperature cooled to 26.3°C by 21 September. A second temperature maximum of 28.4°C was observed on 4 October.

Larval Development and Growth

Larvae were not counted during the culture period. However, it is estimated that at least 75% of larvae died within the first 3 days. Antibiotic concentrations were doubled on the fourth day, after which larval mortality appeared to decrease dramatically. It is not clear whether mortality rates decreased due to increased antibiotic concentrations or decreased larval density. Few mortalities were observed after larvae reached the umbone stage. Mortality rates appeared even lower after larvae reached the pediveliger stage.

Larval development stages are plotted as a function of time in Figure 3. Figure 3 is a semi-log plot because the first week of development—during which *S. tenebrosus* larvae developed through five stages—is emphasized relative to the remaining 7-week culture period—when development progressed through only two stages. (The time periods below refer to time after fertilization.) First cleavage occurred within the first hour, and cleavage stages were observed for up to 6 hours. Blastula stages were observed within 4 to 8 hours. Gastrulation was first observed at 6 hours (5.0% of larvae), and gastrulae were observed until 17 hours (5.0%). Trochophores first appeared within 11 hours (3.3%) and were present in the samples for 40 hours (5.0%). Straight-hinged larvae were first observed in the 21st hour (11.7%), and 8.3% of sampled larvae were straight-hinged on day 10. Umbone-stage larvae appeared within 6 days and were observed until 42 days after fertilization (5.0%). Pediveligers first appeared 12 days after fertilization, and more than 100 were still alive when culture was ended on day 60.

Figure 4 illustrates sample means and standard deviations of larval size (open circles) and developmental stages (filled triangles) as a function of time after fertilization. Three larval growth rate periods were observed. The first period included development from the fertilized egg to the trochophore stage when growth was negative. Mean size decreased from 64.3 to 61.9 μm during this period. The second growth period occurred during development from trochophore to the pediveliger stage. The growth rate during this period (11.4 μm day^{-1}) was the greatest observed over the time series. The average growth rate of pediveliger larvae, the third larval growth period, decreased to 1.0 μm day^{-1}, and growth was asymptotic. An asymptote of ~320 μm was calculated from the fit of a cubic regression of size as a function of time.

Larval Behavior

Larval behavior varied during the culture period. Trochophore through umbone larvae were active swimmers, and were seldom at the bottom of culture vessels

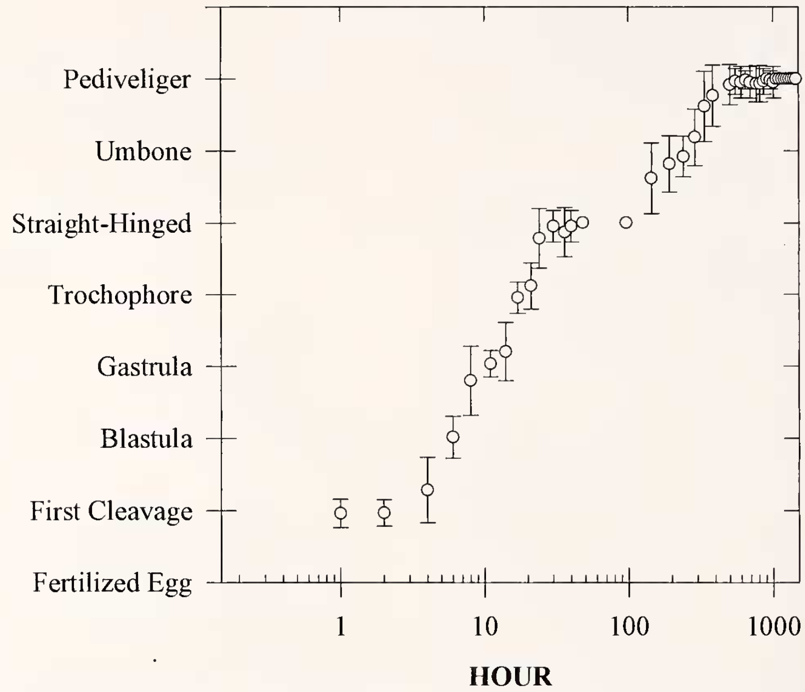

Figure 3. Larval developmental stage as a function of time after fertilization. First week of development is easier to visualize on semi-log plot. Larval developmental stages were numerically coded (see Table 1). Time of first observed settlement is indicated. Error bars are one standard deviation of individuals pooled among larval culture vessels.

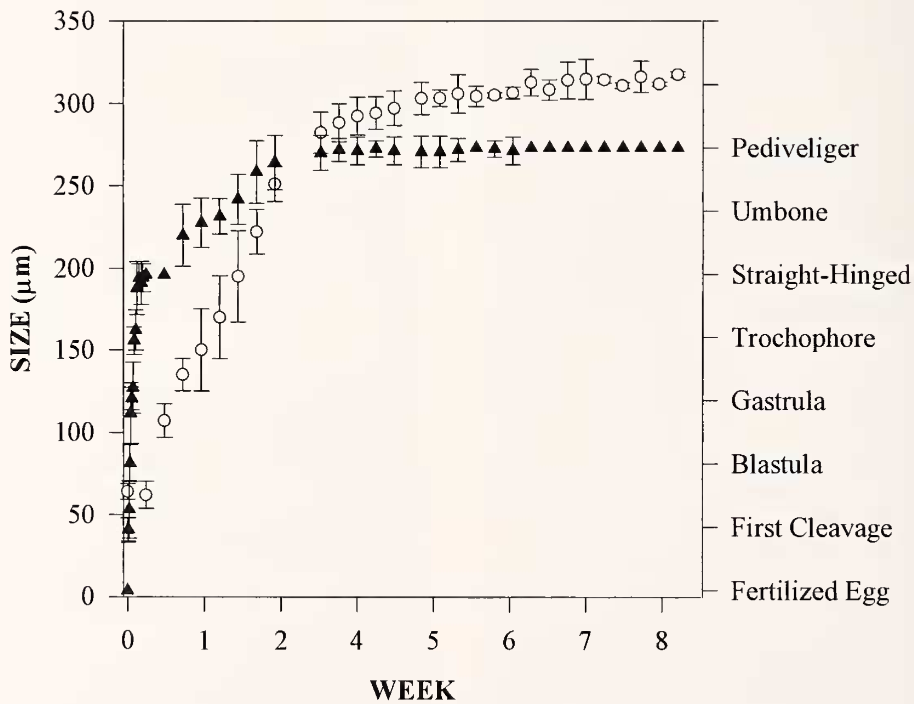

Figure 4. Larval size (open circles) and developmental stage (filled triangles) as a function of time after fertilization. Time of first settlement is indicated. Error bars are one standard deviation of individual larvae pooled among culture vessels.